

Delft University of Technology

TU_Delft

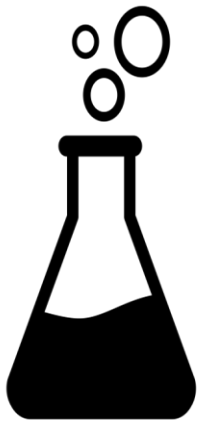


BIO LINK

 TU Delft

Delft
University of
Technology





“Over 60% of **bacterial infections** (and up to 80% of chronic infections) are currently considered to involve microbial growth in biofilms”

Moscoso, M., & García, E. (2009). Pneumococcal biofilms. *International Microbiology*, 12(2), 77-85.





Medical Implants



Air Conditioning
Ducts



Bovine Mastitis



Dental Plaque

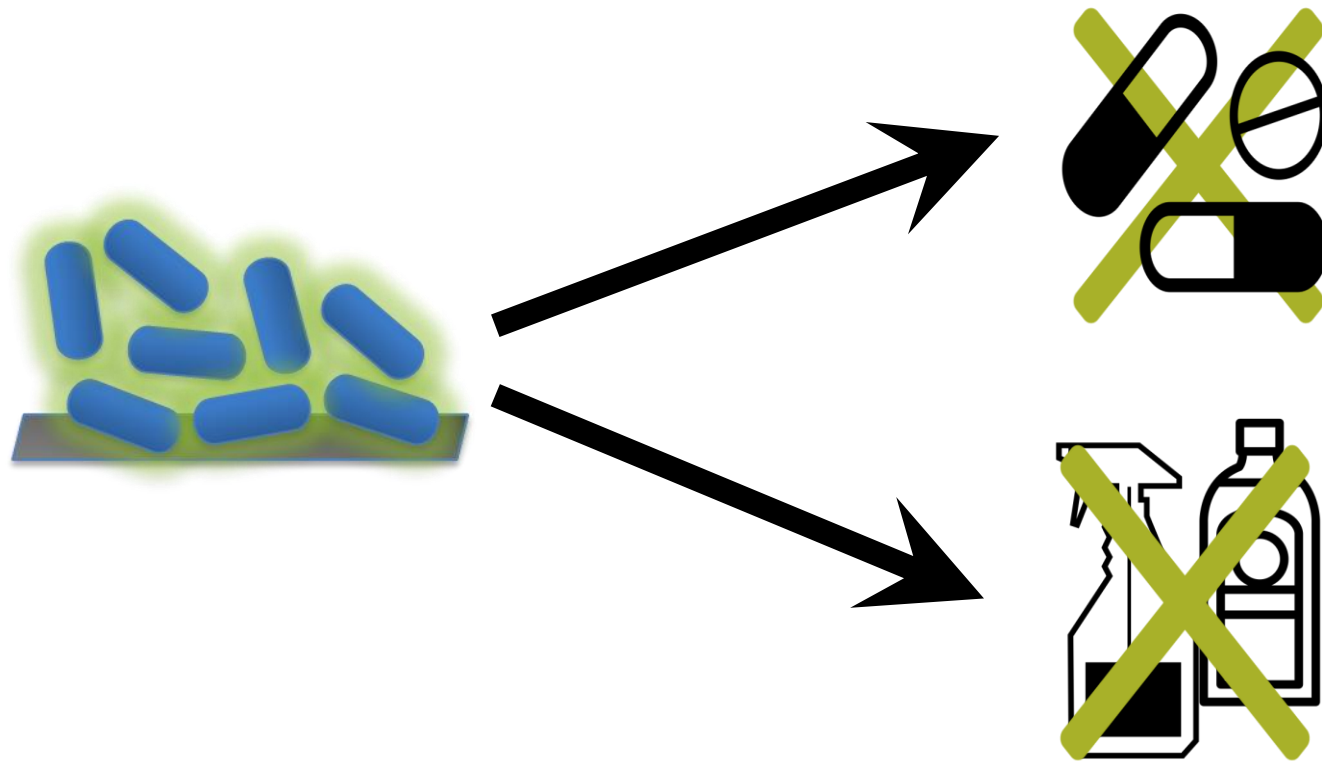


Contact Lenses



Water Pipes

Biofilm resistance



“Little or **no industry standards**, and a profound **lack of awareness** by industrial leaders and society in general”

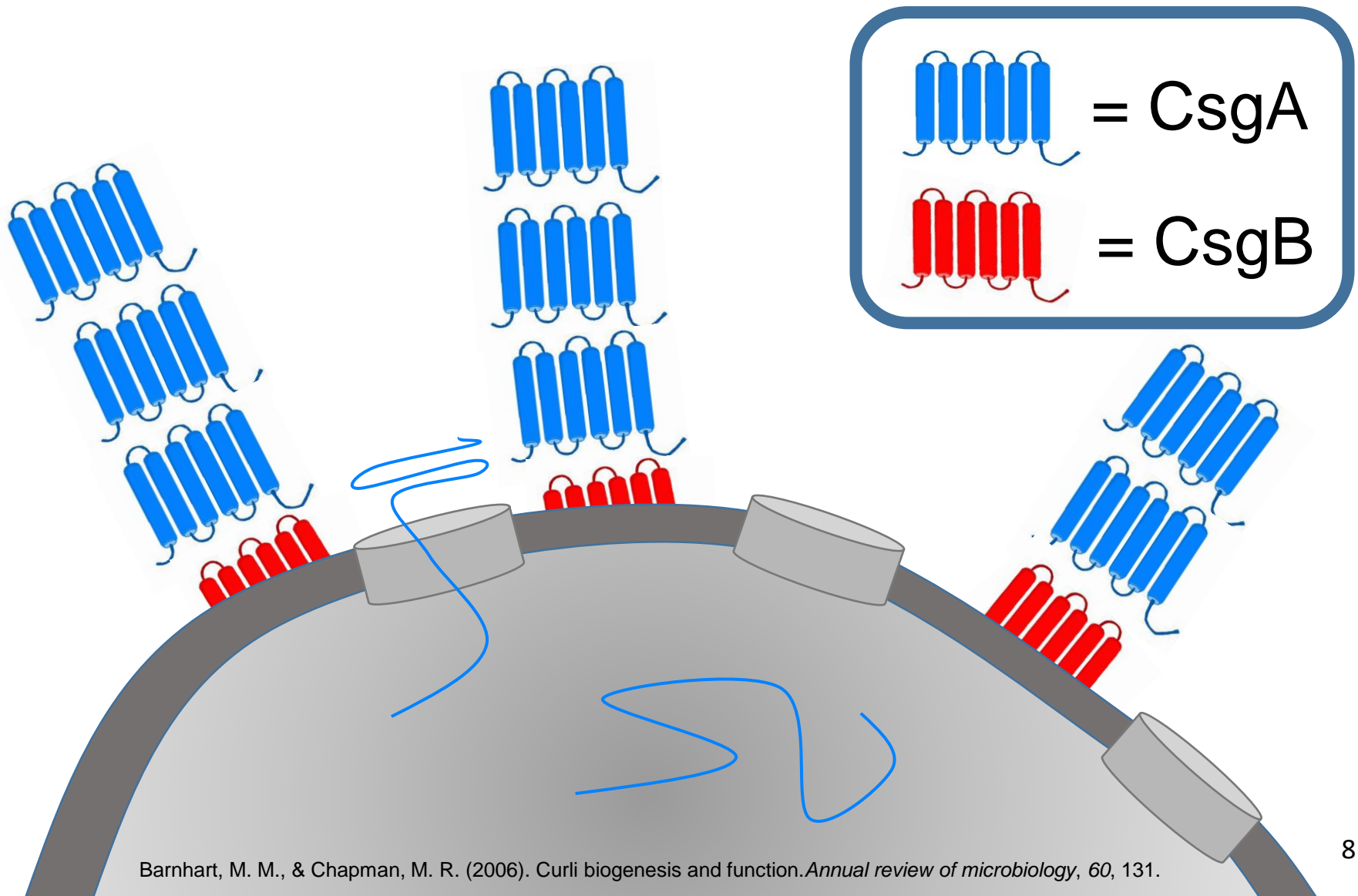
J. Tyler, Tyler Advanced Corrosion Technologies.
Interview - 5th of August 2015



We need a **reliable** and **reproducible**
biofilm test

With current technology this is
impossible

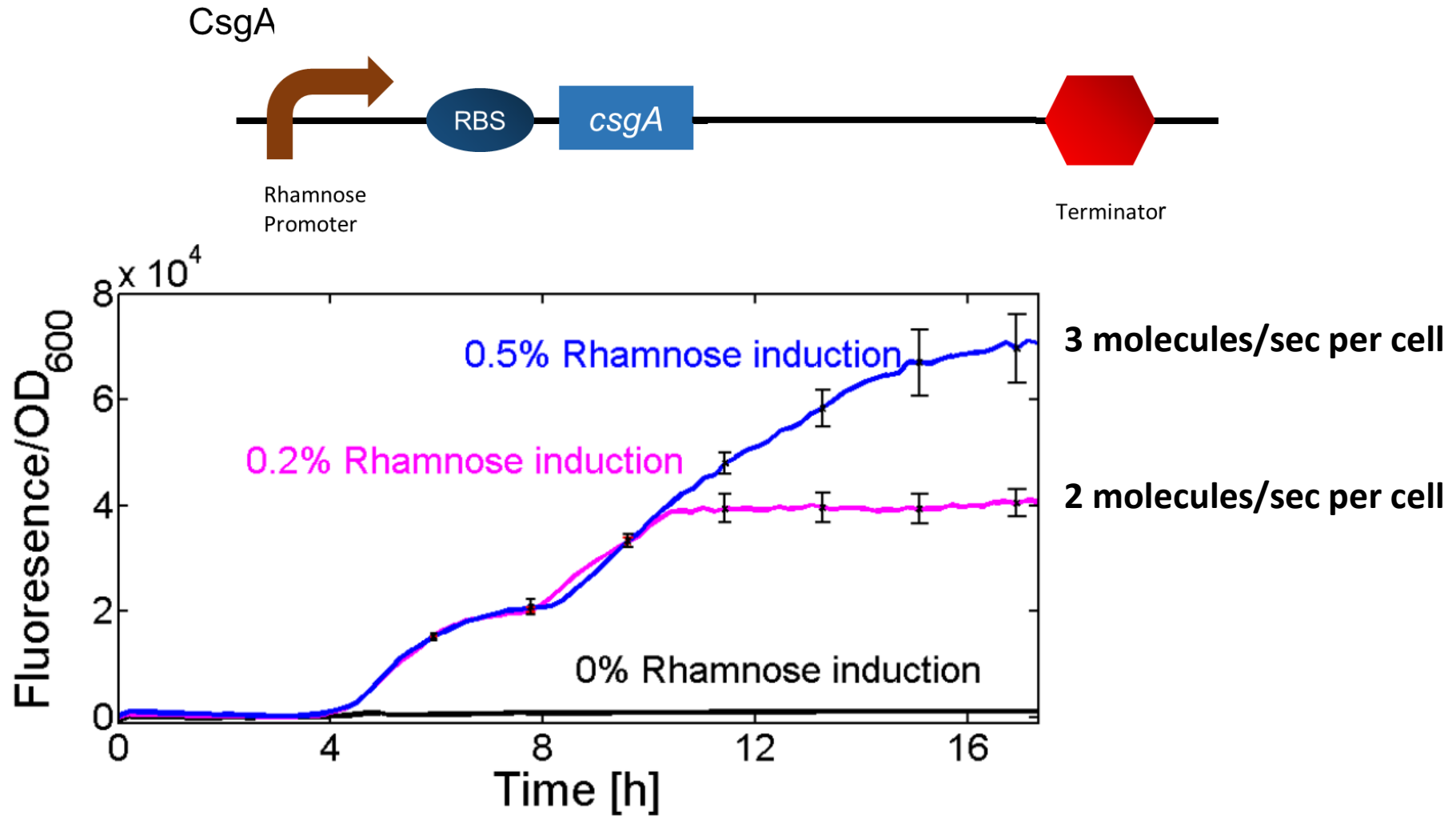
How can we tackle this with synthetic biology?



How can we **control**
nanowire formation?



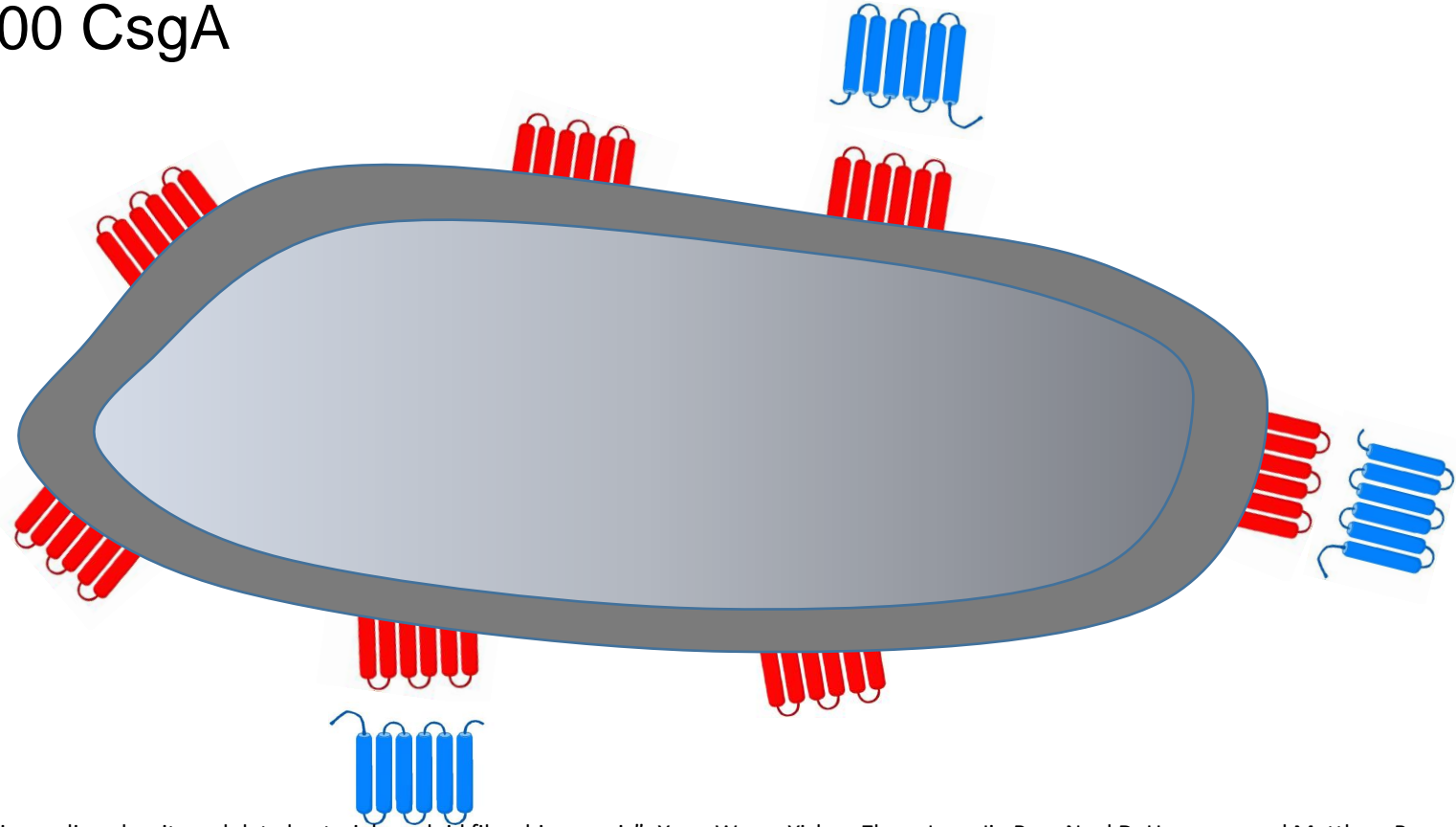
How does rhamnose relate to CsgA production?



How does rhamnose relate to CsgA production?

100-1000 CsgB units/cell

1 CsgB : 500 CsgA



"Gatekeeper residues in the major curlin subunit modulate bacterial amyloid fiber biogenesis", Xuan Wang, Yizhou Zhou, Juan-Jie Ren, Neal D. Hammer, and Matthew R. Chapman, PNAS, (2010), vol. 107., no.1, 163-168



CsgA production is **limiting**
in nanowire formation

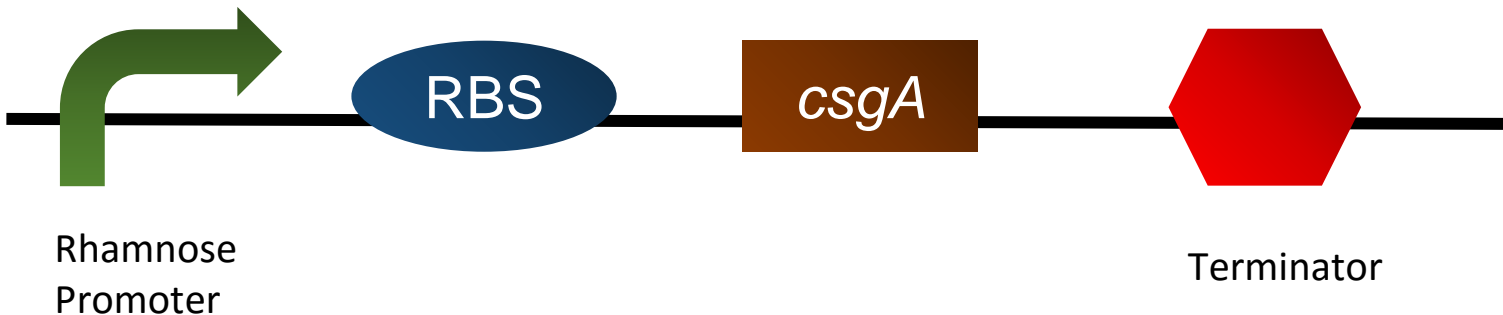


Can we control biofilm formation?

pSB1C3 : Empty (Control)

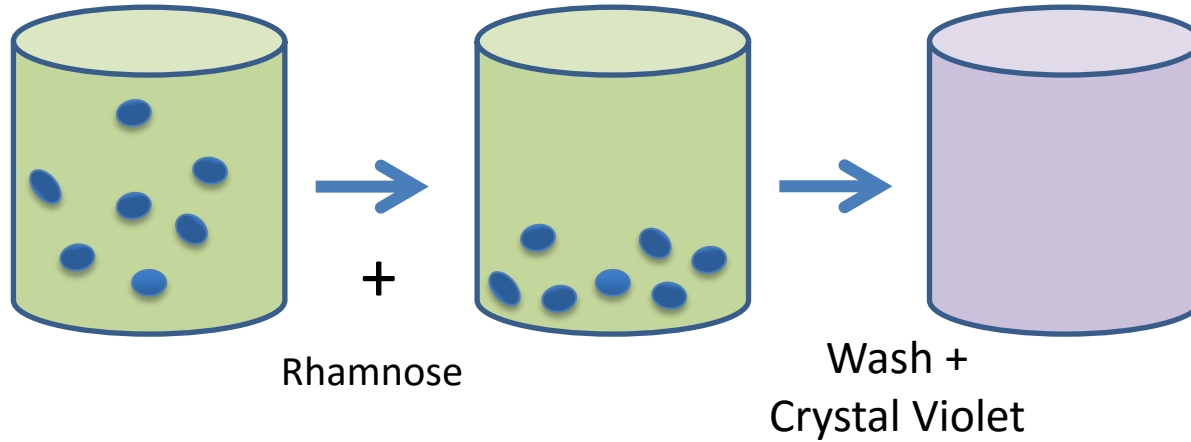


pSB1C3 : CsgA

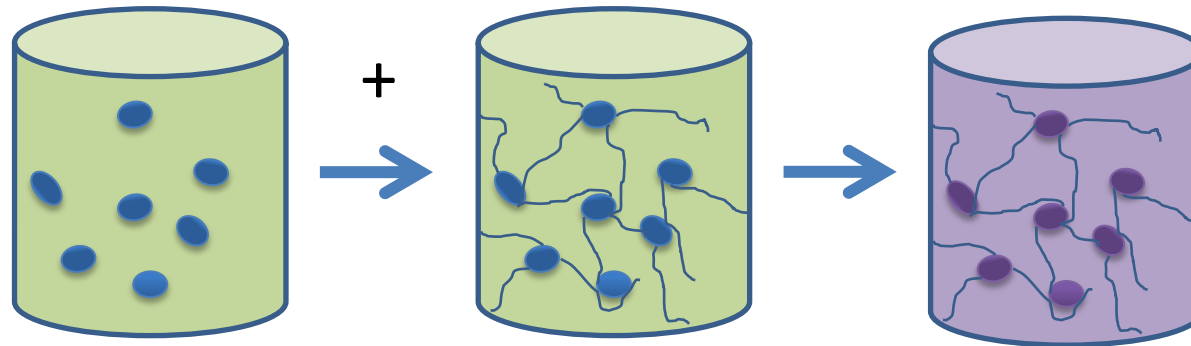


Crystal violet assay

pSB1C3



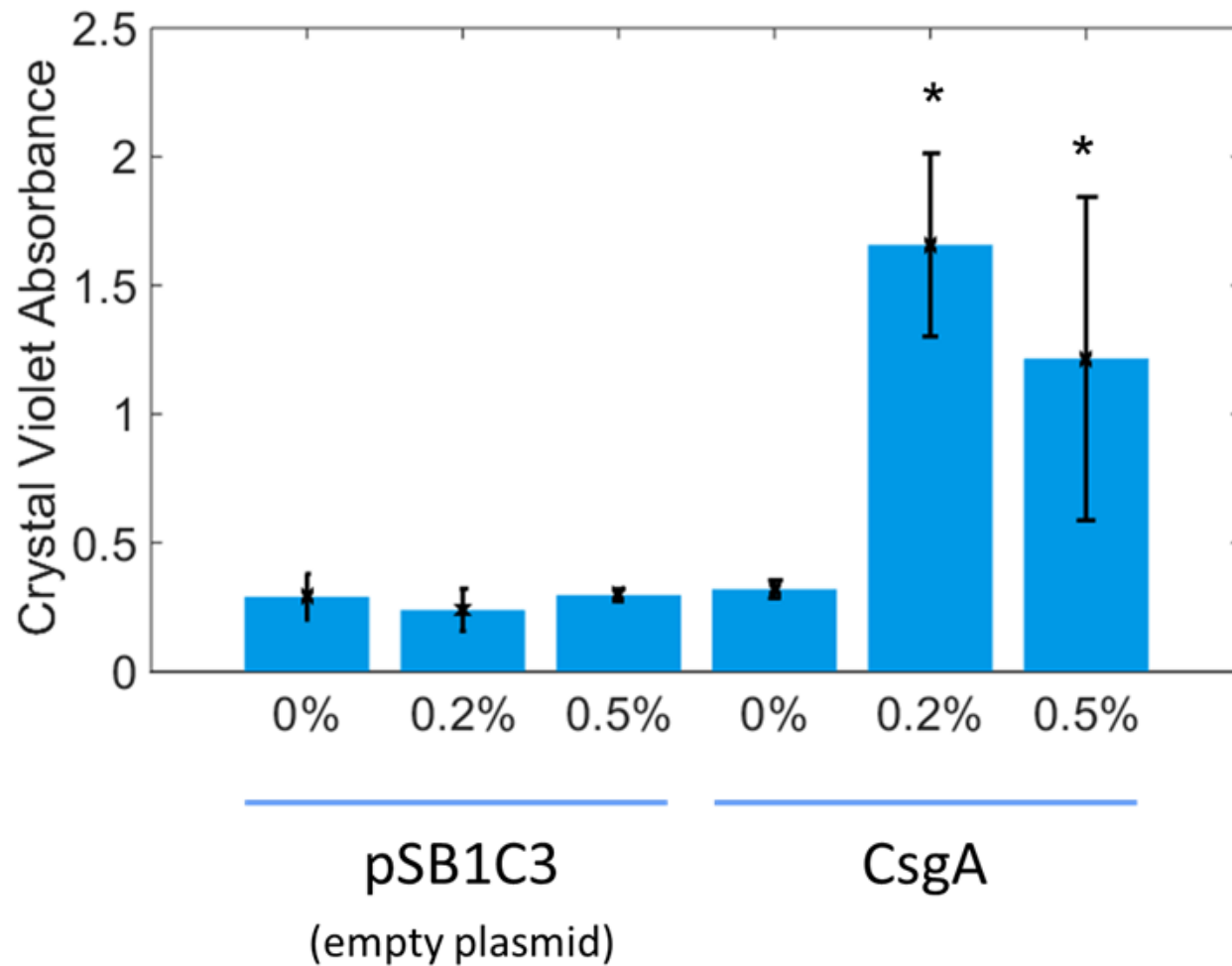
CsgA



Zhou, Y., Smith, D. R., Hufnagel, D. A., & Chapman, M. R. (2013). Experimental manipulation of the microbial functional amyloid called curli. In *Bacterial cell surfaces* (pp. 53-75). Humana Press.



Crystal violet assay

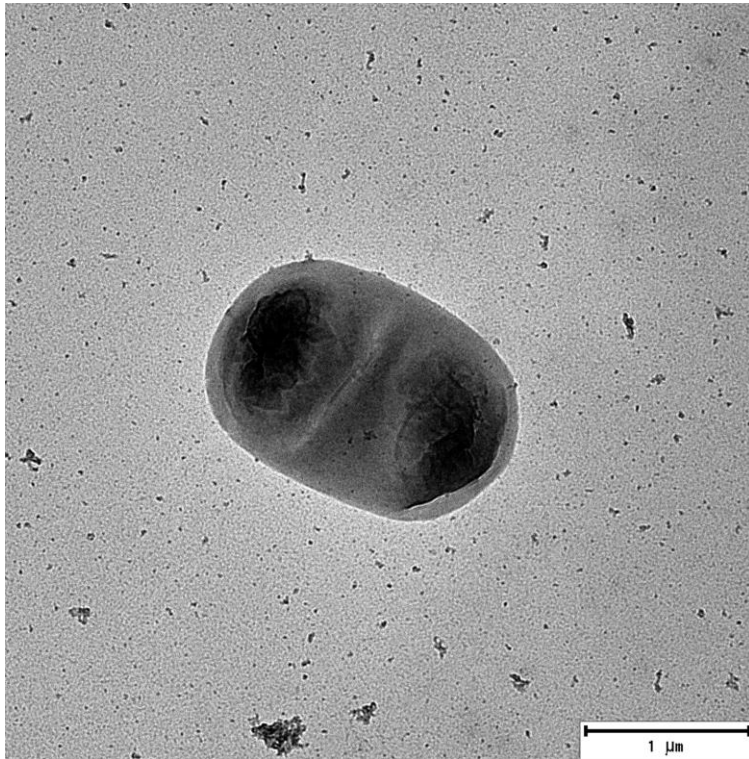


Our engineered cells
produce nanowires forming the
adhesive network of a biofilm

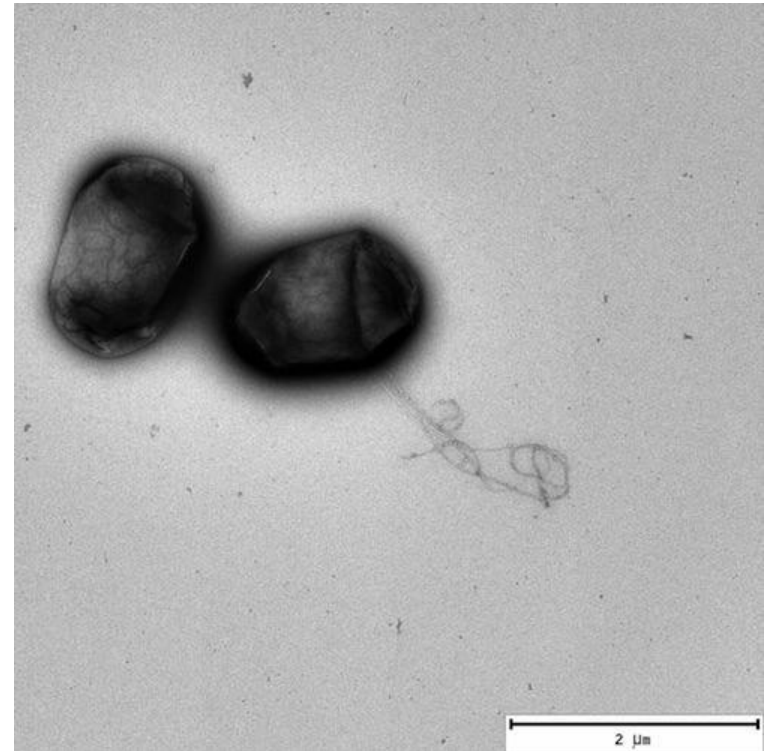


Transmission Electron Microscopy

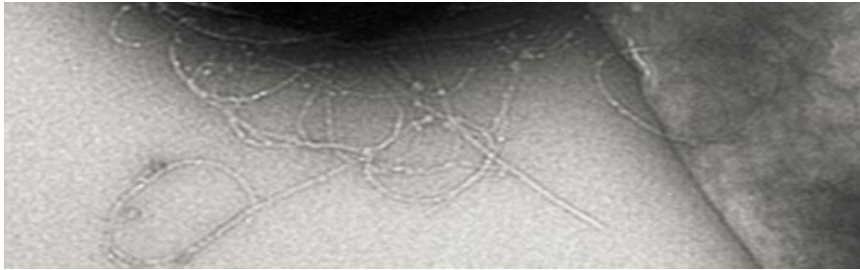
- Rhamnose



+ Rhamnose

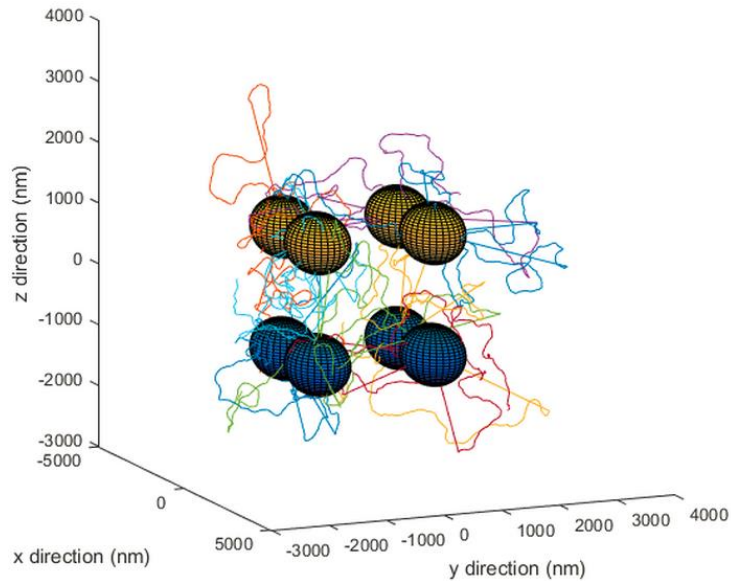


How strong is our biofilm?

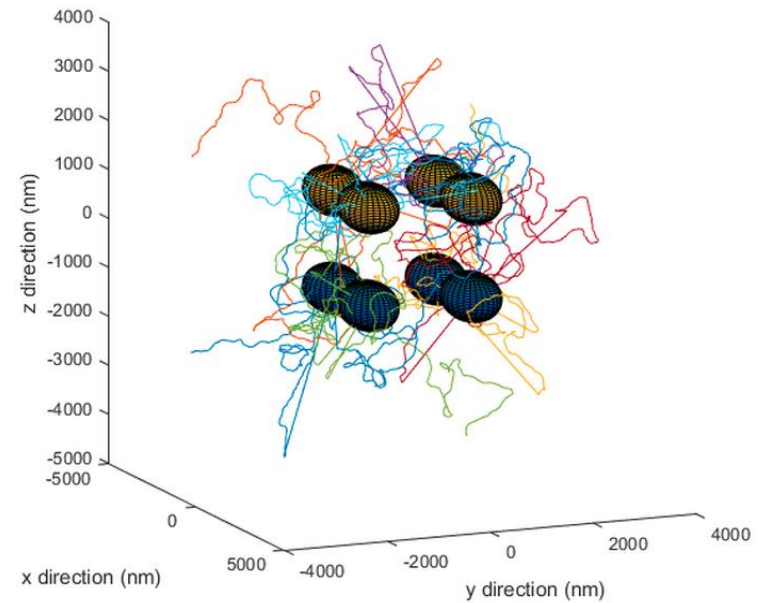


Estimate persistence length

0.2% Rhamnose



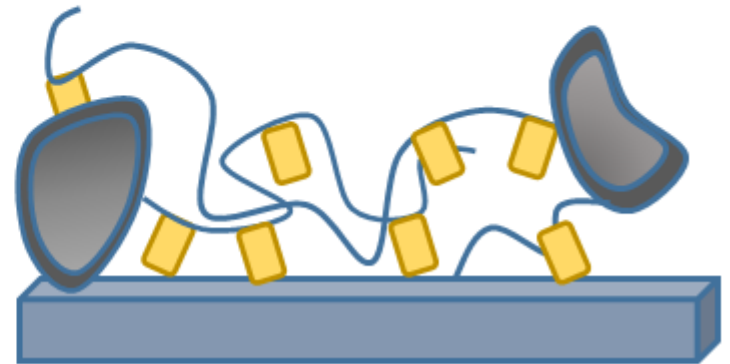
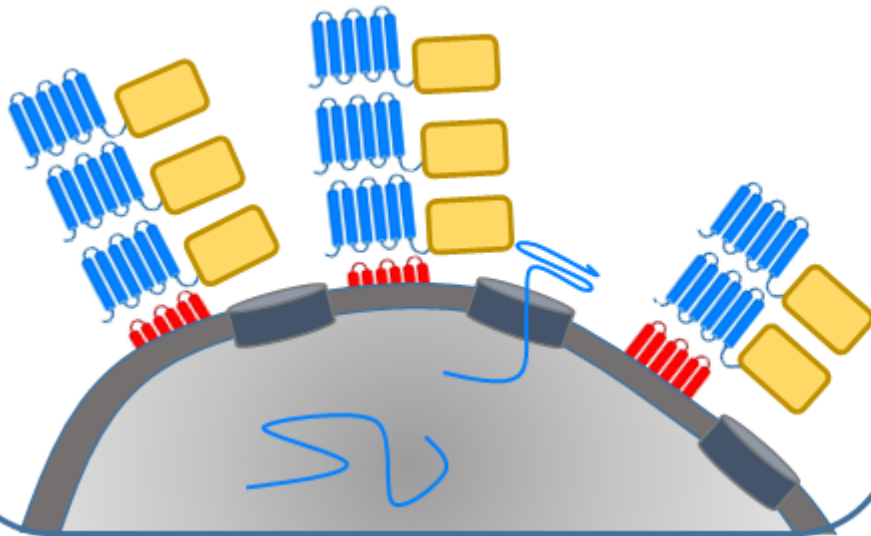
0.5% Rhamnose



Higher rhamnose induction levels lead
to a **stronger biofilm**



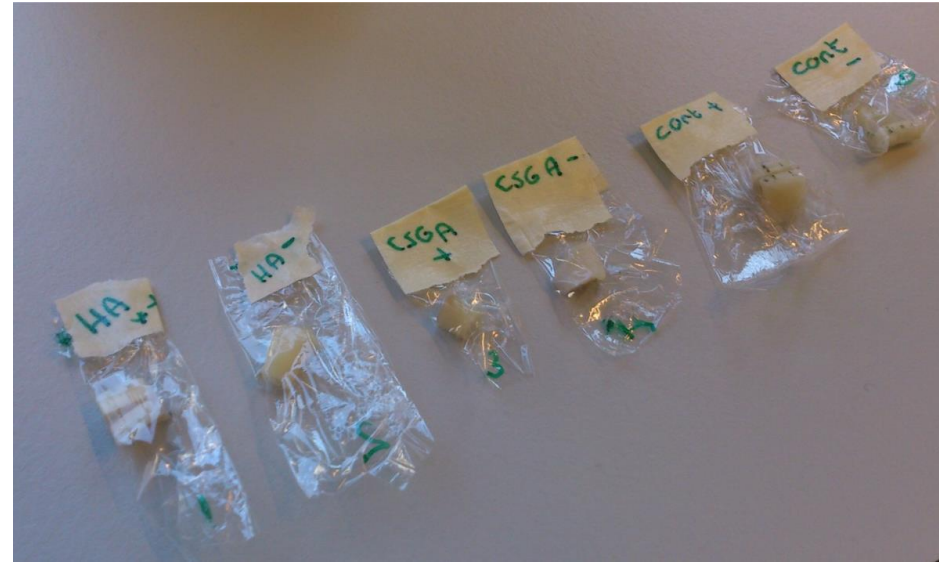
Can we make the biofilm stick?



Nguyen, P. Q., Botyanszki, Z., Tay, P. K. R., & Joshi, N. S. (2014). Programmable biofilm-based materials from engineered curli nanofibres. *Nature communications*, 5.

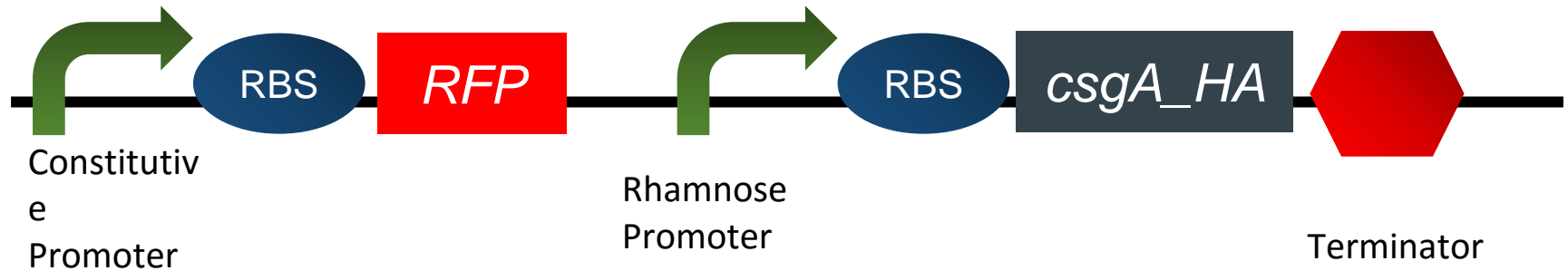
Zhong, C., Gurry, T., Cheng, A. A., Downey, J., Deng, Z., Stultz, C. M., & Lu, T. K. (2014). Strong underwater adhesives made by self-assembling multi-protein nanofibres. *Nature nanotechnology*.

Hydroxyapatite affinity

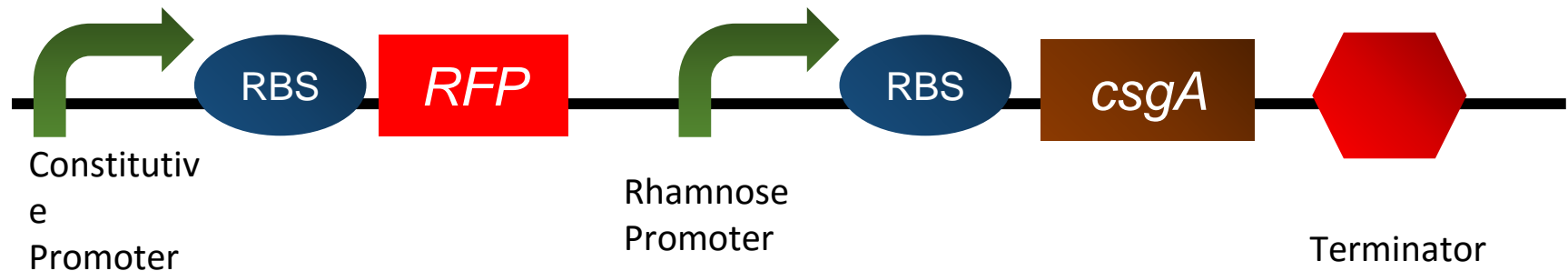


Hydroxyapatite affinity

pSB1C3 : CsgA_HA_RFP



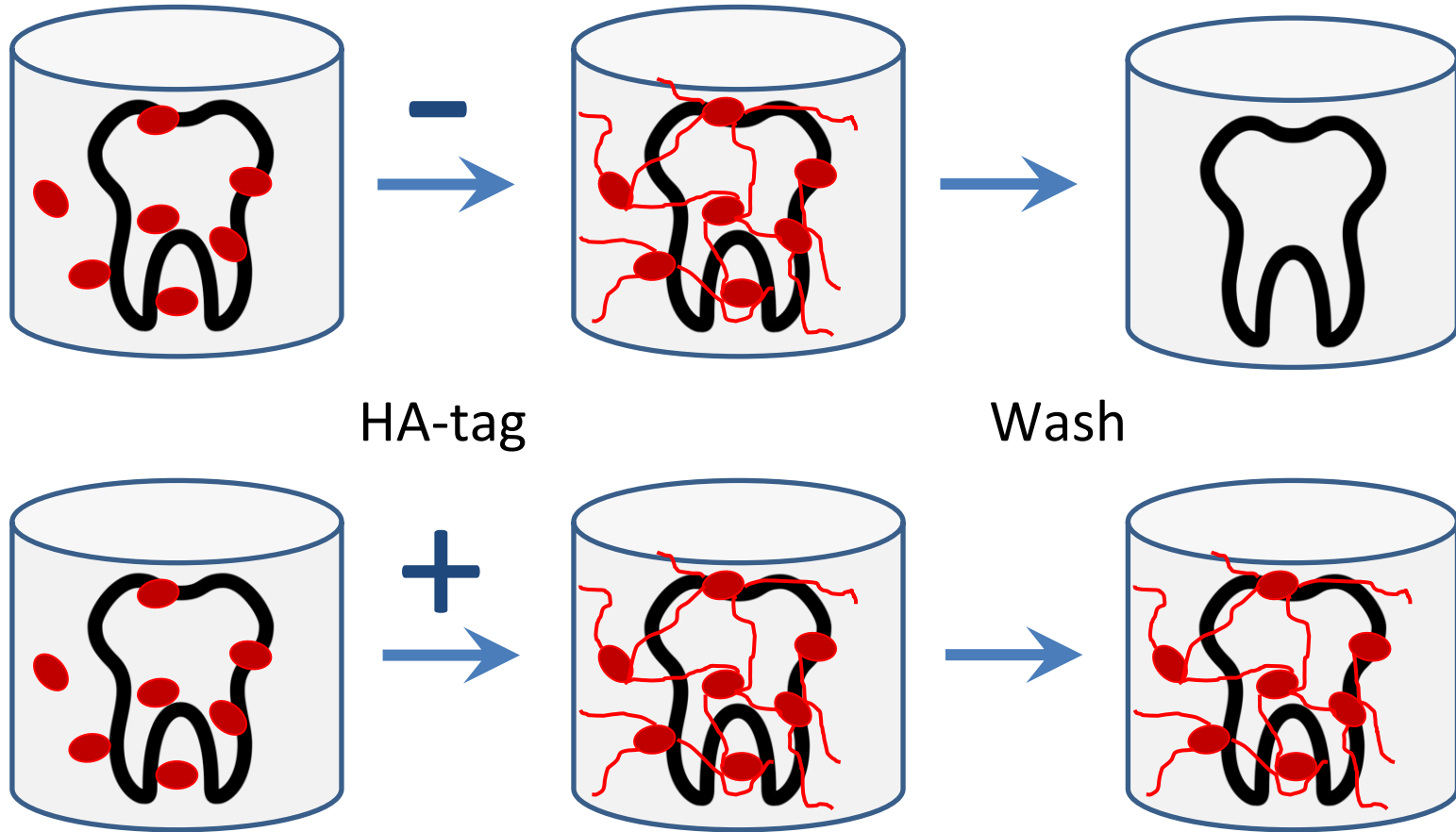
pSB1C3 : CsgA_RFP



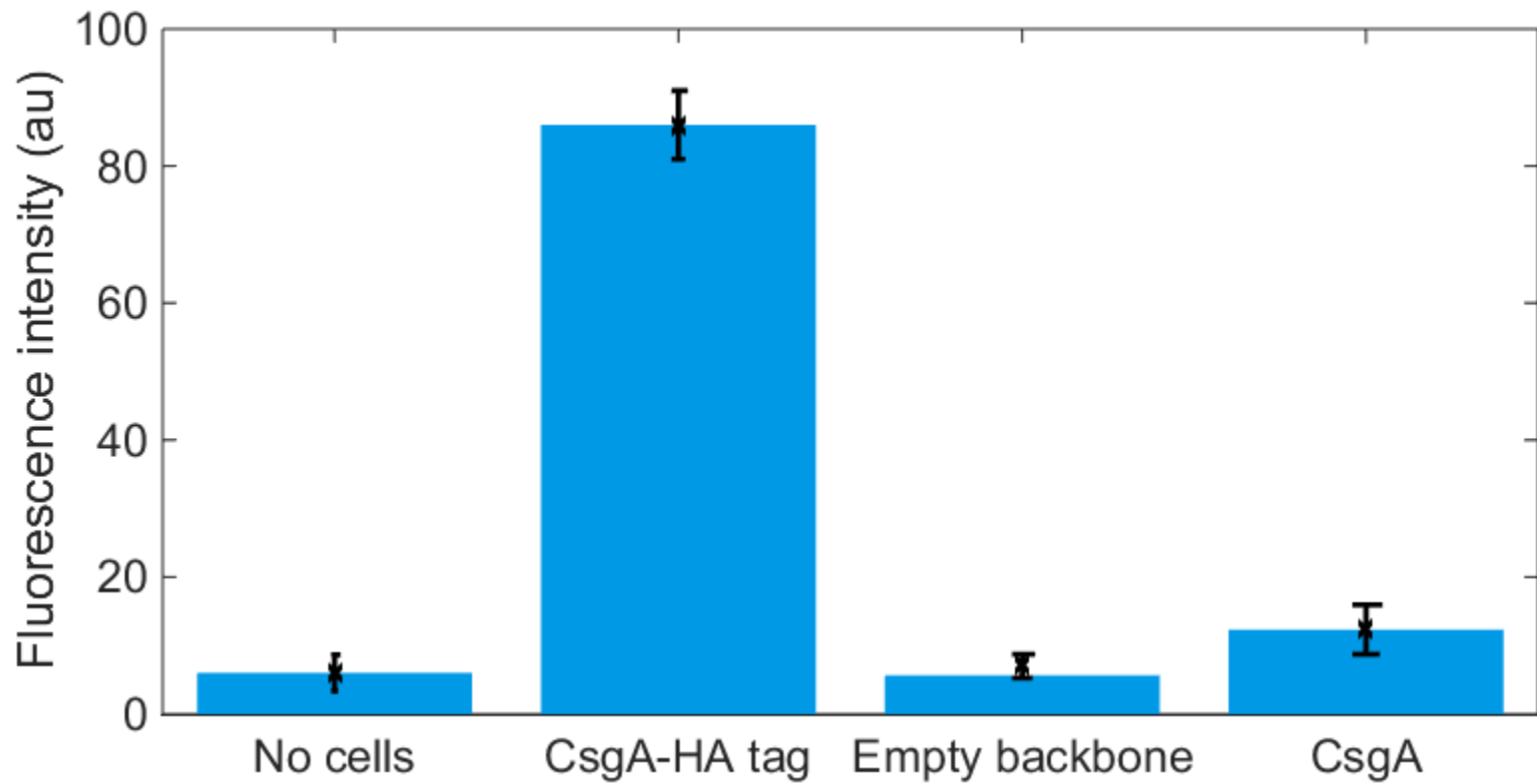
M. Roy, S. Stanley, E. Amis, M. Becker, "Identification of a Highly Specific Hydroxyapatite-binding Peptide using Phage Display", *Adv. Mater.*, **2008**, 20, 1830-1836.



Hydroxyapatite affinity



Tooth affinity assay



Our affinity tag is able to **increase cell adhesion** of biofilms to teeth



“The currently used biofilm models allow only for **flat** or **simple curved** surface areas.”



Dr. Marko de Jager,
Principal Scientist Oral Healthcare,
Philips Research



Can we actively **control** the shape
and composition of a biofilm?



How do we add more control to printing?



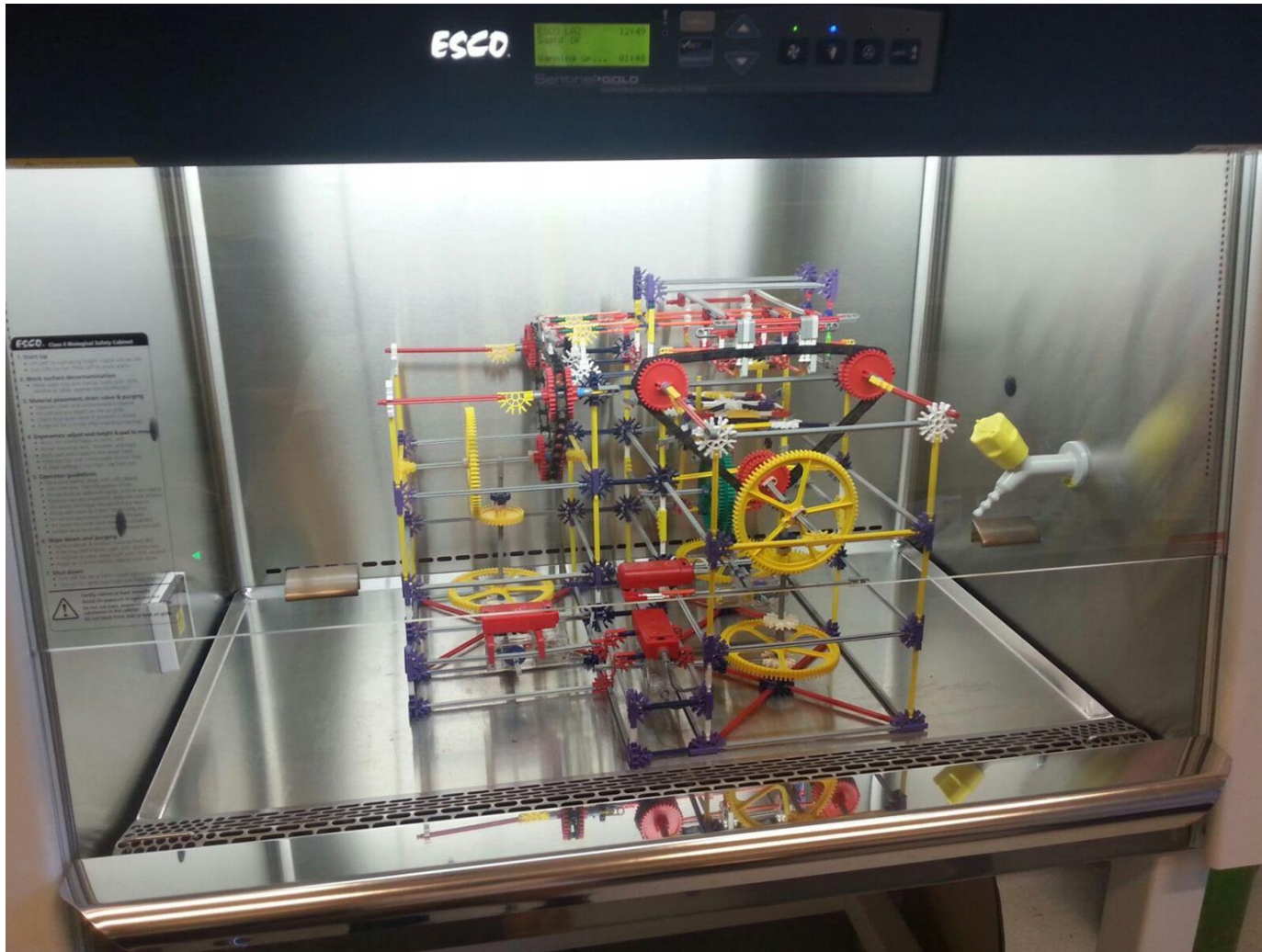
KNEX - Building our own 3D printer



Building our own 3D printer - The BIOLINKER



The Biolinker

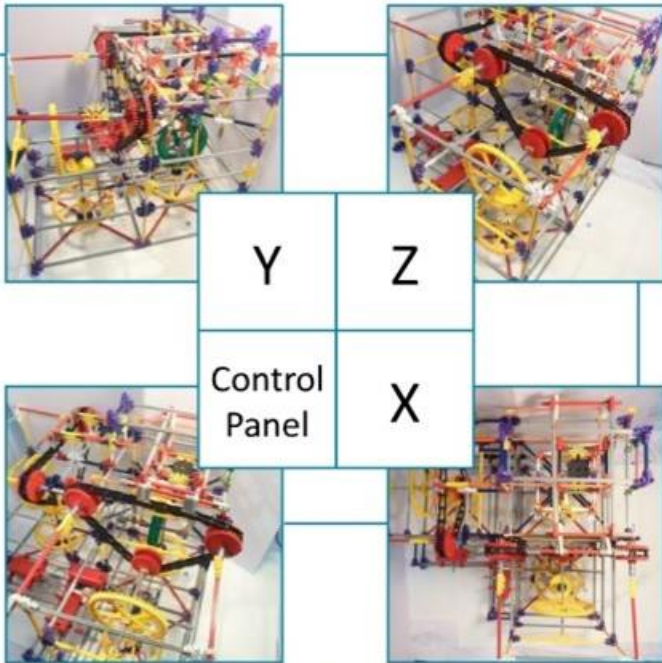


BIOLINKER - a DIY Open Source Printer

BIOLINKER MANUAL

The iGEM 2015 of Delft University of Technology, BIOLINK, has strived to find a way to create a structured biofilm. The solution they came up with is BIOLINKER, a 3D printer made of K'NEX. After trying out several designs, the model you're about to build turned out to be the best suitable for their cause.

This final construction consists of 4 area's, called x, y, z, and control panel. Each area has it's own main function, since the printer can move in three directions. The area's x, y, and z indicate a certain direction of movement. The control panel is where the movements can be controlled, because this is where the motors and on/off switches will be located. Throughout the manual, we will refer to these directions so the middle figure shown below is useful to keep in mind.



K'NEX: 291 €

Pump: 250 €

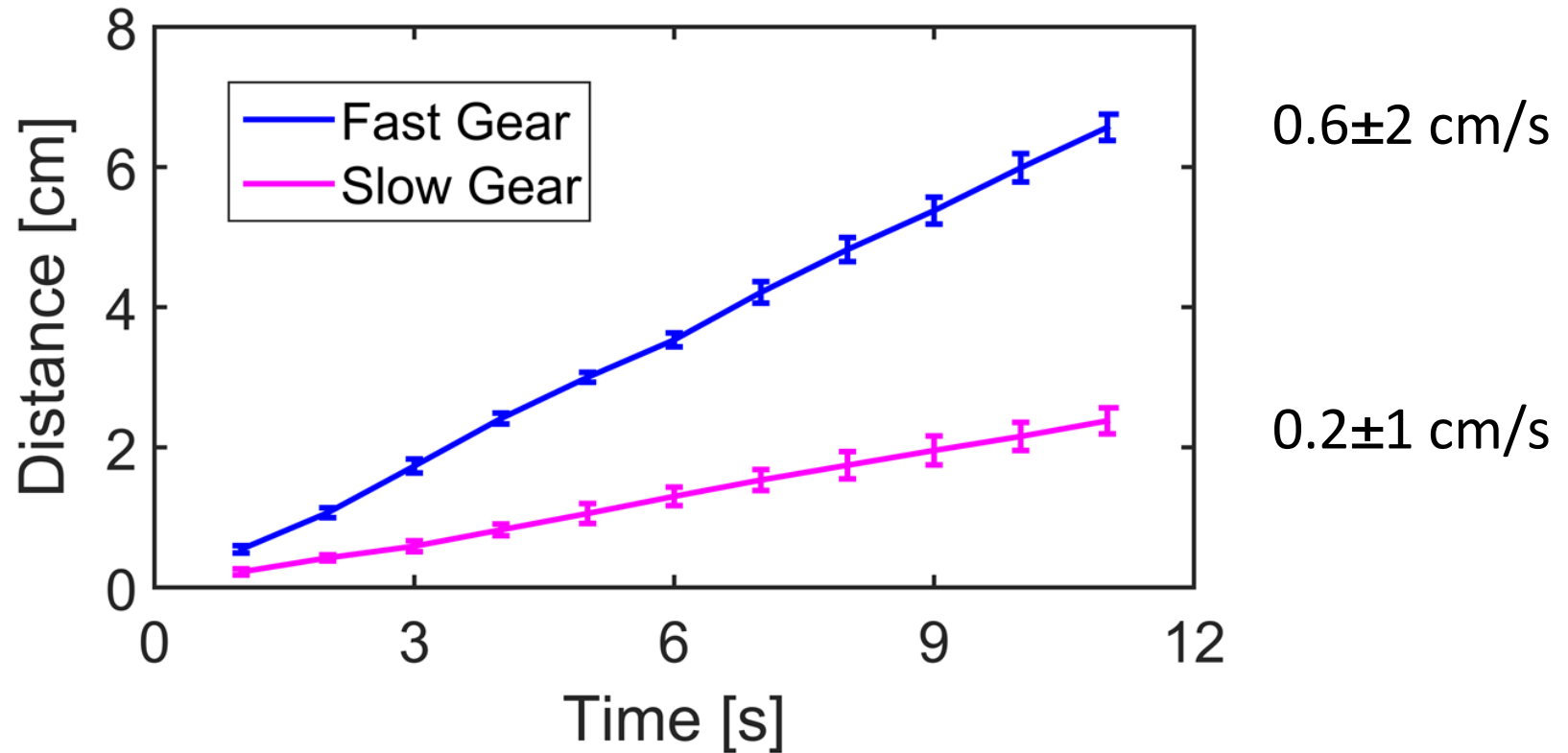
Total: 541 €

Cheapest 3D
Bioprinter:

5000 €



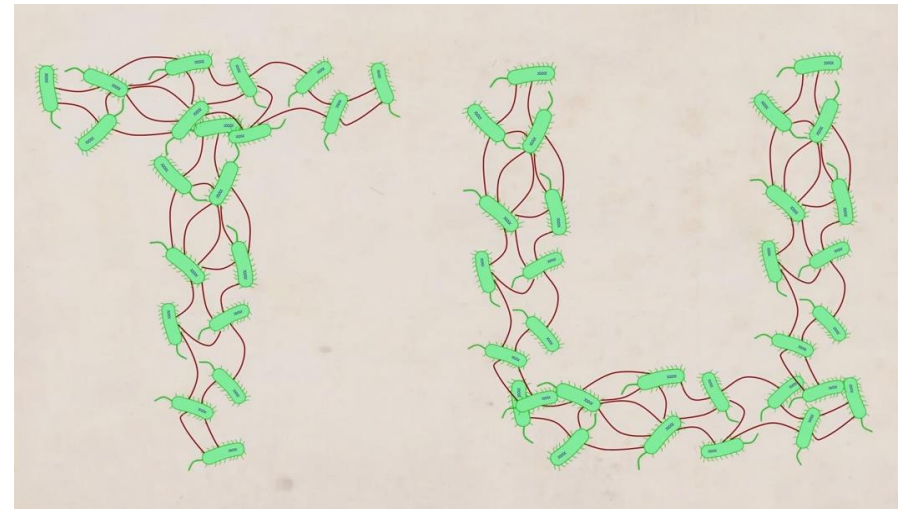
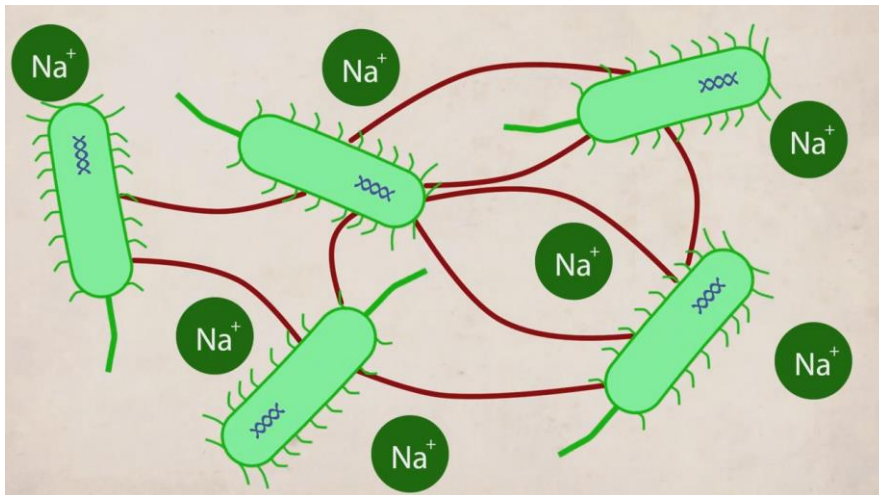
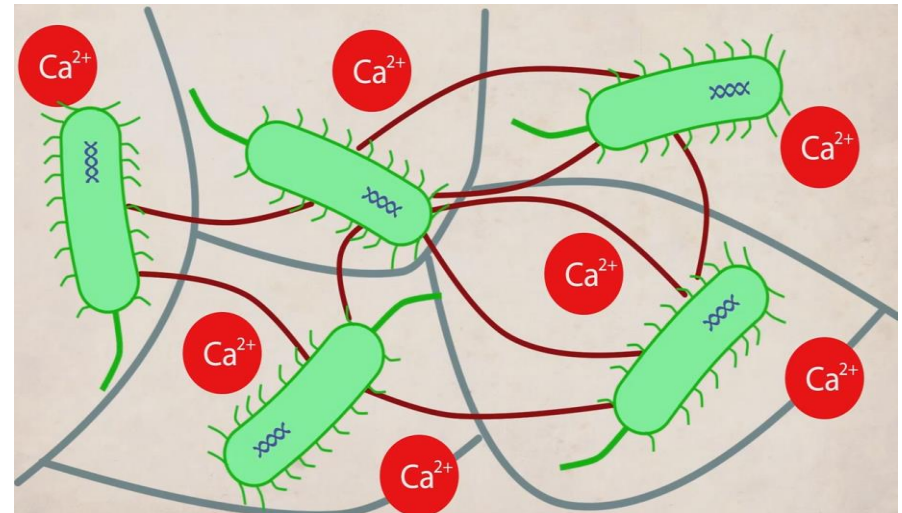
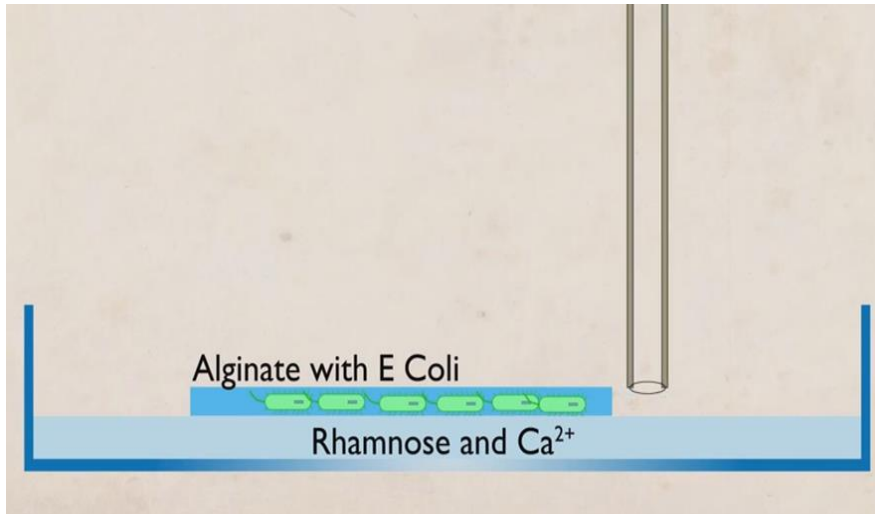
BIOLINKER Velocity



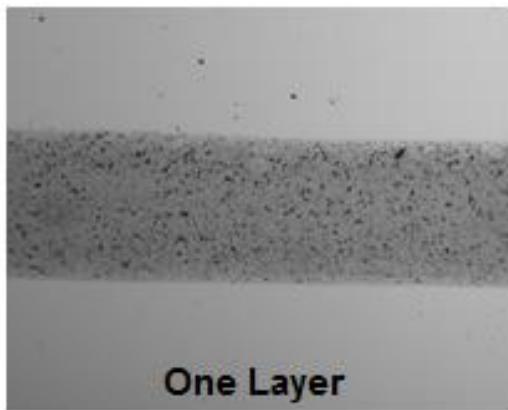
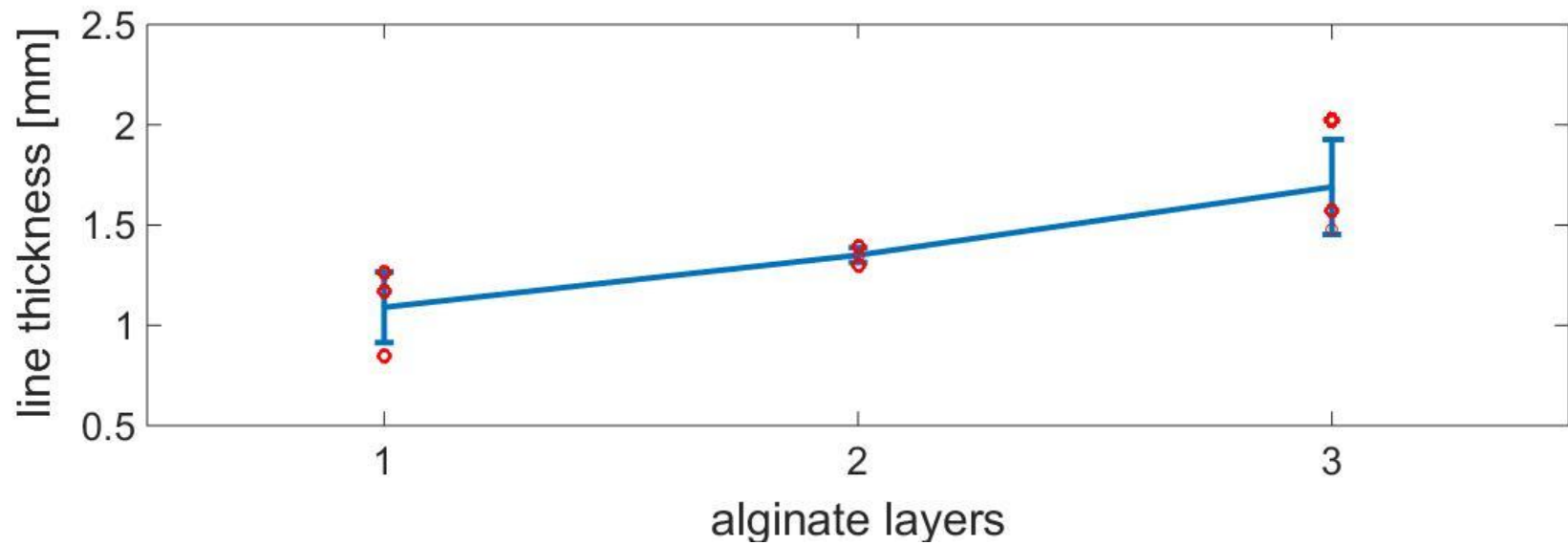
We made a customizable **DIY**
3D-printer, that is **safe, inexpensive** and
easy to build



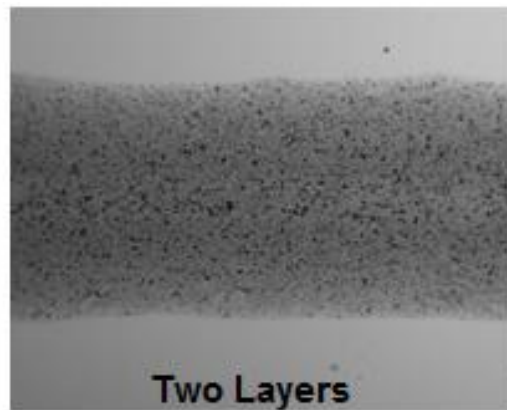
How do we use our customized bioink?



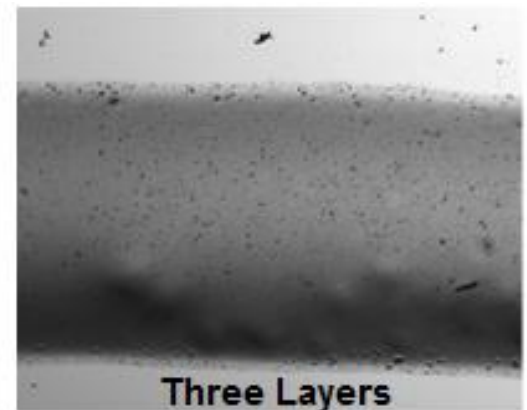
Alginate thickness



1.09 mm

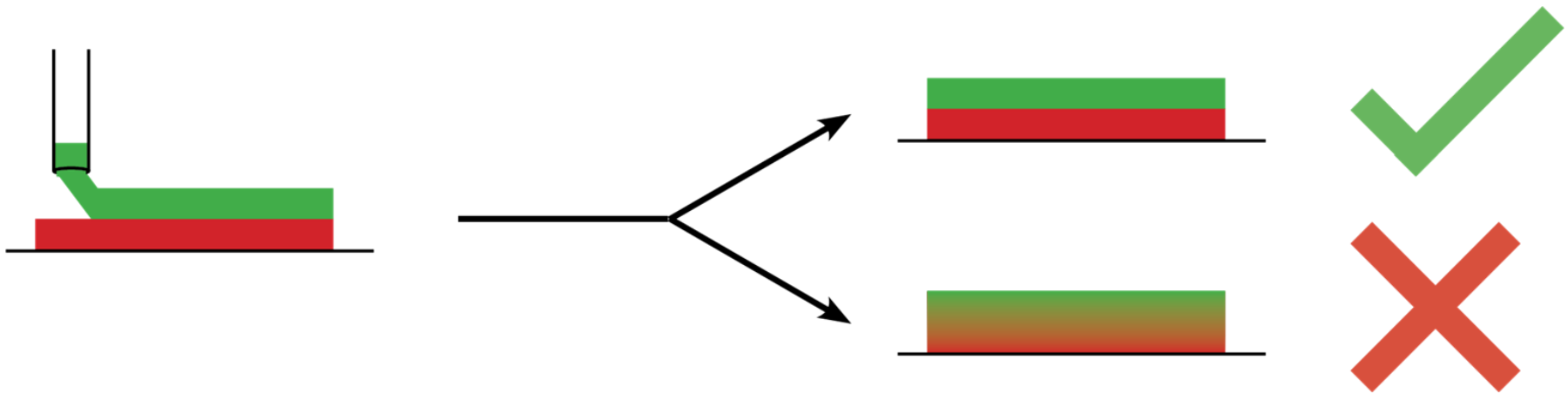


1.35 mm



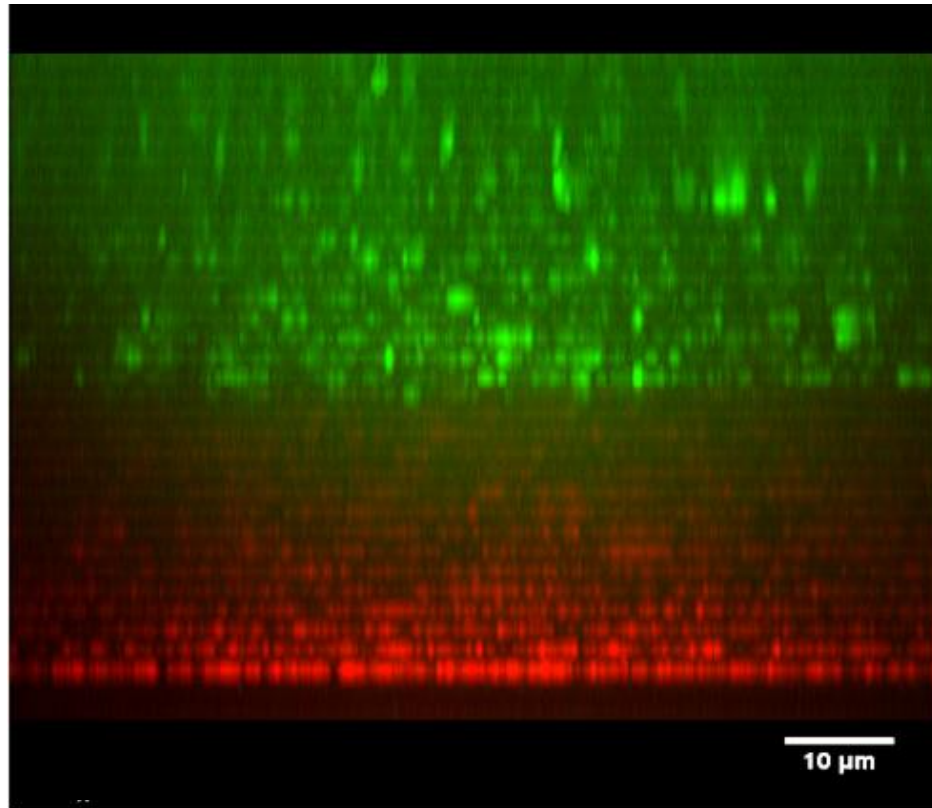
1.69 mm

Can we print separate layers?

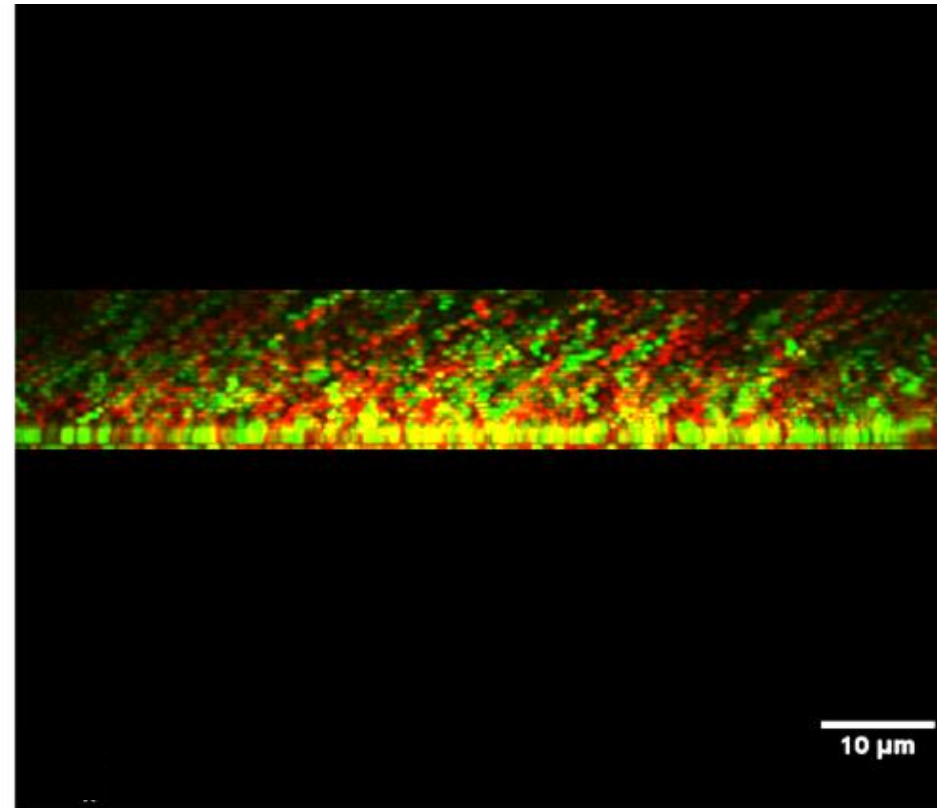


Can we print separate layers?

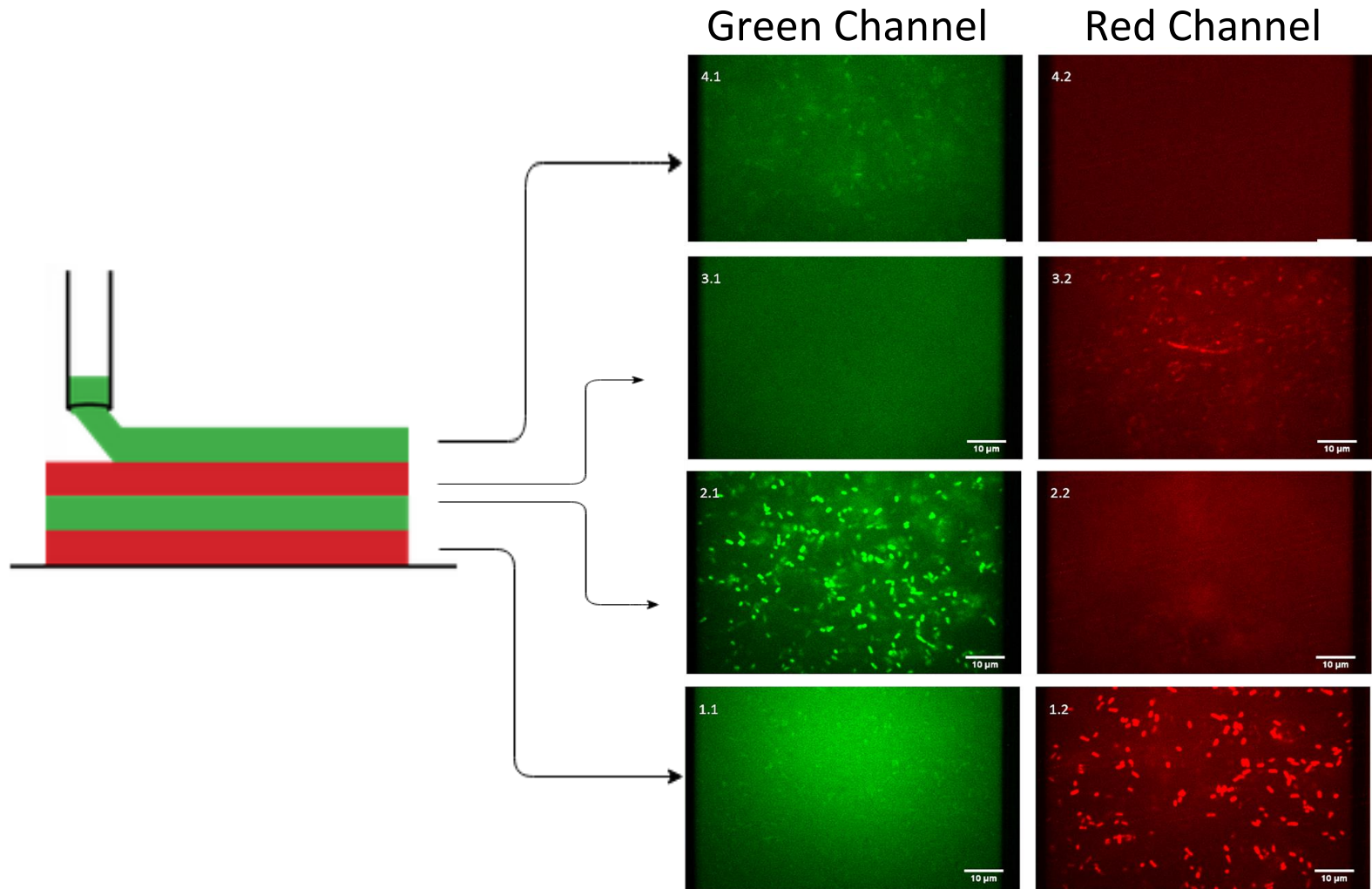
+ Alginate/ CaCl_2



- Alginate/ CaCl_2



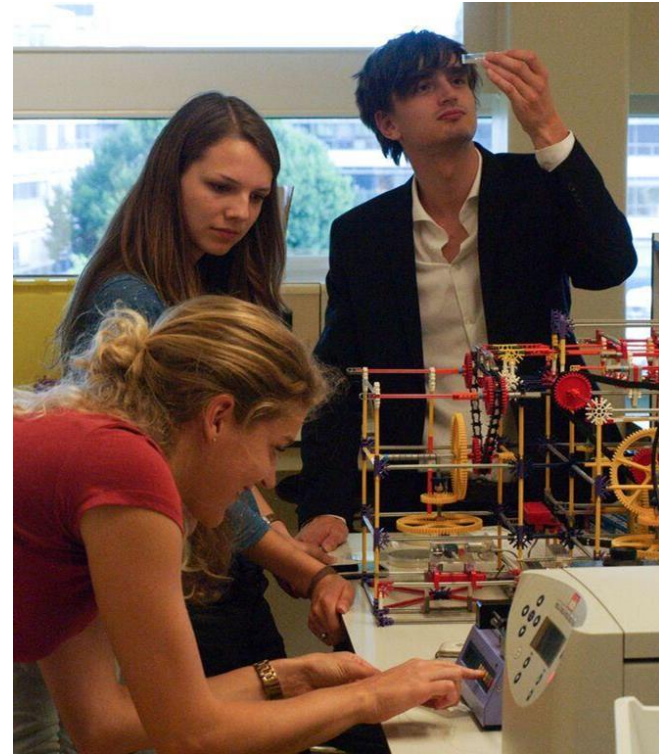
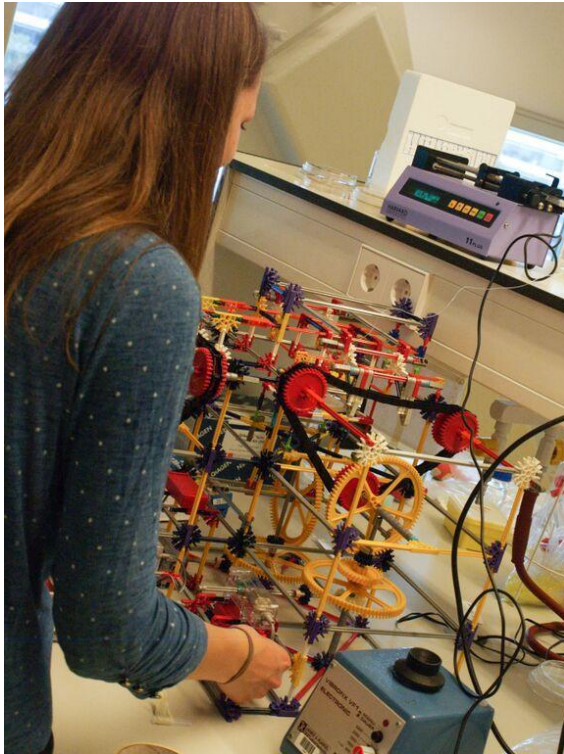
Can we print separate layers of bacteria?



We can print **structured layers** of different bacteria and control their **spatial distribution**



What about printing with other types of bacteria?



Groningen Collaboration

- *Bacillus subtilis*
- Platform technology



Cooperations for new applications



Berlin



KU Leuven



Amsterdam



What do experts think?

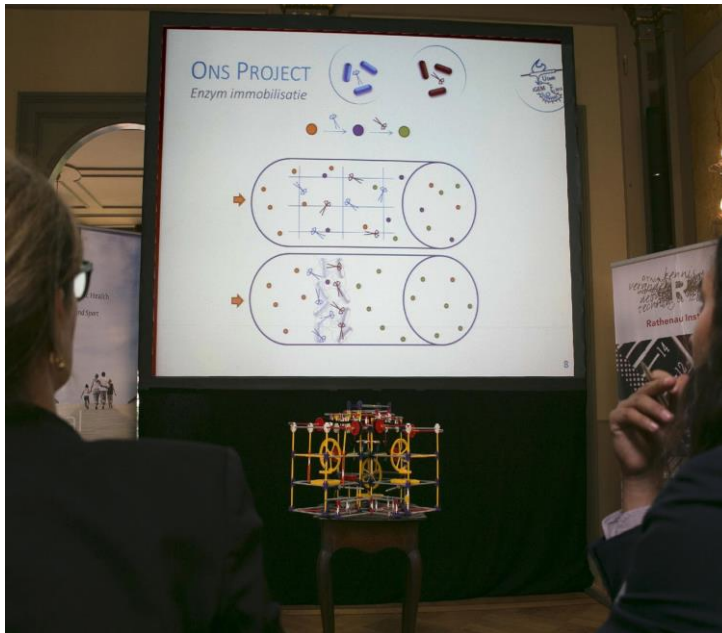


Discussion about ethics of synthetic biology



National Institute for Public Health
and the Environment
Ministry of Health, Welfare and Sport

dy nam i k e n n i s s e n g
v e r a n d e r i n g
w e t e n s c h a p p e l
d e b a t t e
t e c h n o l o g i e
Rathenau Instituut



National Institute for Public Health and Environment
Rathenau Institute



Policy and Practice Tool



Sharing our project with the community



Youth

Tour to collect K'NEX donations



Students

Business Case



General Public
A Day of Wonder



Conclusions



Cheap, open source and **user friendly**.
Promoting **DIY** in Synthetic Biology.



CsgA production is **limiting** in biofilm formation
Rhamnose induction levels **control** biofilm formation.



Our cells **produce** nanowires forming the **adhesive**
network of a biofilm
Affinity tag **increases** cell adhesion to teeth



Excitement from **society, students** and the **government**.
Business plan exploring market potential
The **P&P Tool** improves **ethical** and **safety** discussions

Attributions

Team Members

- Anne Rodenburg
- Hector Sanguesa Ferrer
- Liana Uilecan
- Marit van der Does
- Max van 't Hof
- Michelle Post
- Samantha Basalo Vazquez
- Stefan Marsden
- Tudor Vlas

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- Jorine Eeftens
- Timon Idema

